HNPCC: Clinical Validity

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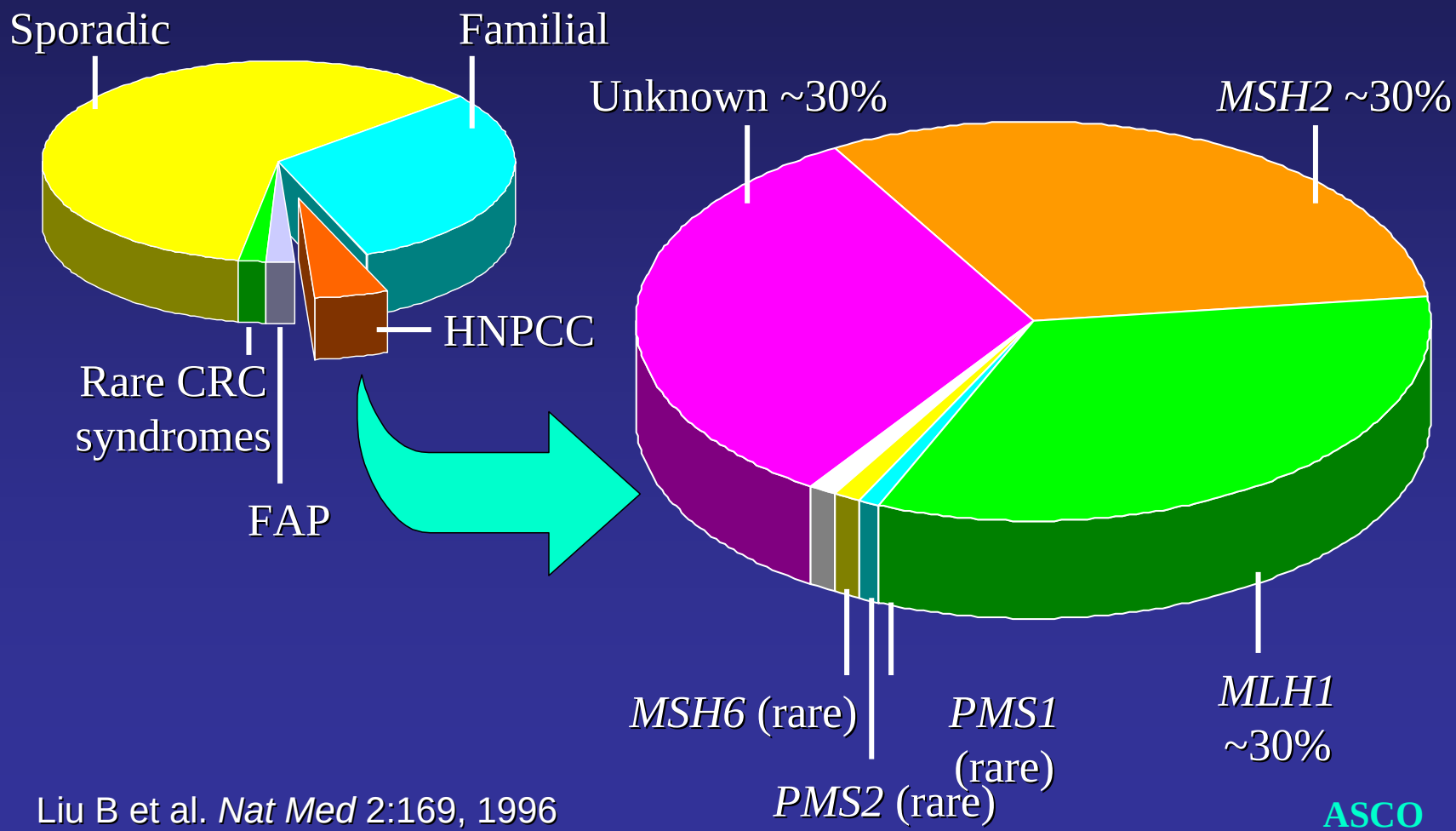
Disorder & Setting

- Definition: HNPCC = Hereditary Non- Polyposis Colorectal Cancer, AD
- Clinical finding: Families, early onset, multiple cancers, tumors right-sided, better survival, more common in males, extracolonic tumors
- Clinical setting: Identify patient & test relatives, preventative; Amsterdam/Bethesda criteria
- DNA Tests: MSI, IHC, sequencing
- Screening Questions: Pedigree analysis

Contribution of Gene Mutations to HNPCC Families



Liu B et al. *Nat Med* 2:169, 1996

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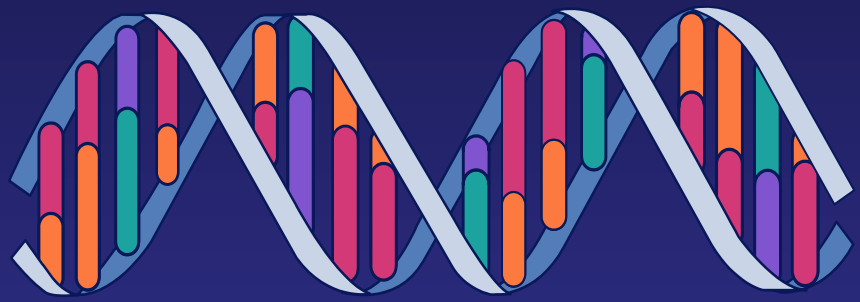
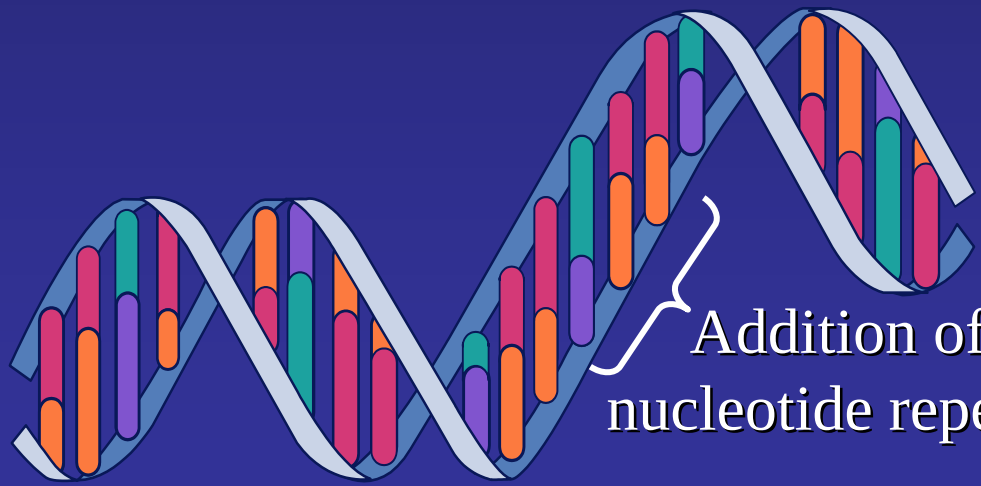
Mismatch Repair Failure Leads to Microsatellite Instability (MSI)

Normal



Microsatellite instability



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Clinical Sensitivity of Microsatellite Instability Testing

- MSI in tumors of most HNPCC patients that fulfill Amsterdam criteria (~90%)
- 80-95% HNPCC tumors are MSI-H positive
- Germline mutations in MMR genes associated with MSI-positive tumors (94%)
- Overall ~90% clinical sensitivity for MSI
- Advantage of MSI: Exclude HNPCC w/o sequencing MMR genes
- NCI Standard Panel of 5 markers recommended
- Limitation of MSI: Requires tumor sample

Clinical Specificity of Microsatellite Instability Testing

- Aaltonen ‘98: 85% clinical specificity, with 15% sporadic CRC tumors MSI-high
- De la Chapelle ’03:
  - 12% patients MSI-H
  - 25% of MSI-H had HNPCC mutations
  - 60% mutations identified by sequencing
  - Estimate ~7% false positive rate
- Overall data consistent with ~10% false positive rate for MSI testing, or 90% clinical specificity

Clinical Sensitivity of DNA Tests in 59 HNPCC Families

		Clinical Sensitivity (%)	
DNA Test	**Num (%)**	**Observed**	**Adjusted***
MLH1, MSH2	35 (59)	59	53
MSH6	3 (5)	64	58
Southern Blot	14 (24)	88	78
No Mutation	7 (12)		
All	59 (100)		

* Reduced by 10% to account for proportion of mutation positive individuals who are negative for MSI

Clinical Sensitivity of Mutation Testing in Unselected CRC Patients

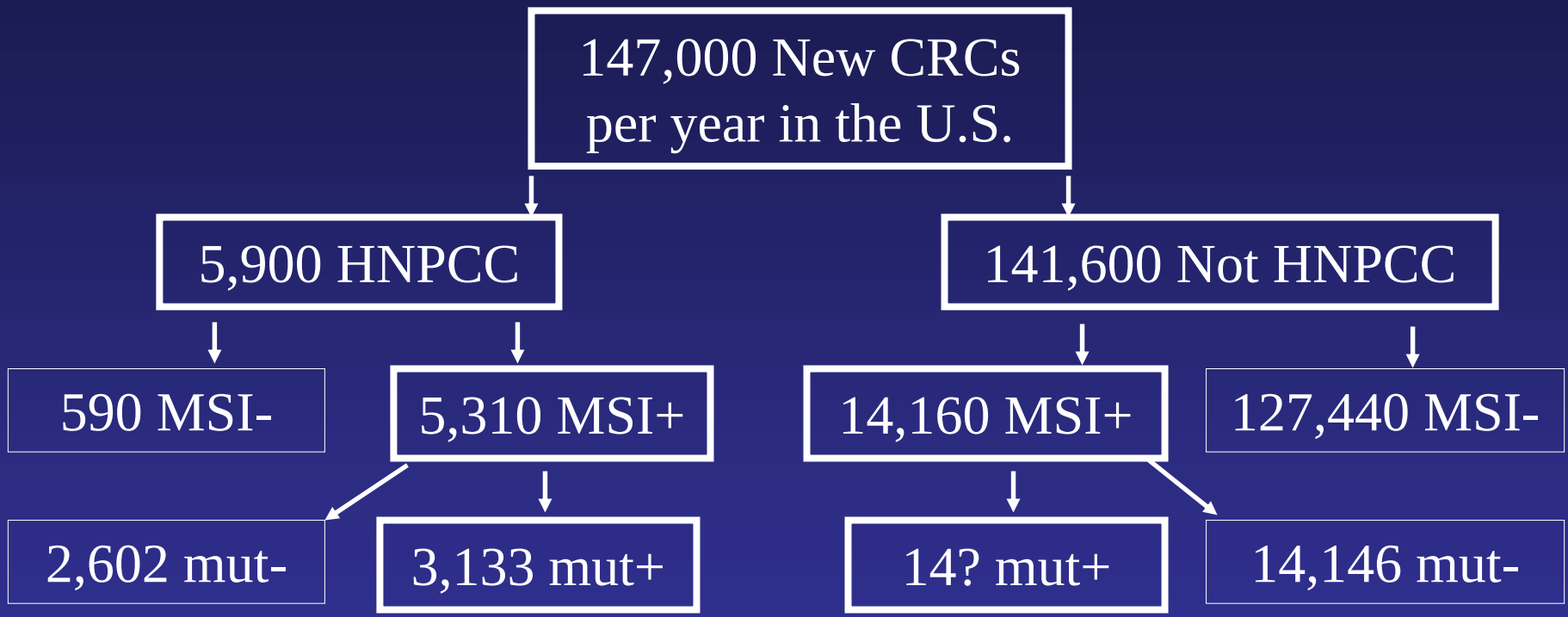
- All CRC patients in defined geographic region are studied
- de la Chapelle study in Ohio
  - 12% CRC patients had MSI-High tumors
  - 25% of these had mutations in MLH1, MSH2, or MSH6
  - Protocol includes MSI testing of all CRC patients, and mutation analysis for patients with MSI-High tumors.
  - Estimates nationwide program could detect 21,000 carriers of HNPCC mutations in first year of testing.

de la Chapelle Am J Hum Genet 72:236, 2003.

Clinical Specificity of Mutation Testing in Unselected CRC Patients

- How often does a false positive result occur?
- Only if analytical result is incorrect, or if test interpretation is incorrect (missense mutations).
- Both events are uncommon, so clinical specificity is likely to be high, but specific estimate not available.
- Assume false positive rate of 1 per 1000 (99.9%)

Screening for HNPCC in in Newly Diagnosed Cases with Colorectal Cancer



PPV = 3,133/(3,133 + 14) = 99.6% (diagnostic?)

NPV = 14,147 / (14,147 + 2,602) = 84%

Analysis performed similar to de la Chapelle (2003) and Ramsey (2001).

Alternative Protocols Avoids MSI Testing in All Patients

- MSI testing only if the patient's family history satisfies the Amsterdam or Bethesda criteria
  - Difficult to establish sensitivity and specificity
- Wijnen ('98) proposed formula for calculating probability of MMR mutation in CRC family using family history & age of diagnosis
  - May be useful for diagnosed CRC patients
  - Limitations: Relies on Amsterdam criteria; uses Dutch/Norwegian data
  - No method for estimating probability of finding a mutation in an HNPCC suspect (similar to BRCA and breast cancer) and is considered a **Gap in Knowledge**

Are there methods to resolve clinical false positives in a timely manner?

- Labs offering testing are aware of interpretation issues with missense mutations.
- Analytic false positives are low (unknown).
- Analytic true positives are common in this test setting.
- Routine re-testing of patients with positive DNA results is unlikely to be useful.

What is the prevalence of the disorder in this setting?

- De la Chapelle (2003) estimates 3% of all CRC patients have germline mutation in *MLH1*, *MSH2*, or *MSH6*.
  - 12% of consecutive CRC cancers are MSI-H & 25% of MSH-H have MMR mutations
  - Assumes MSI test has negligible false negative rate in HNPCC & neglects contribution of genes other than *MLH1*, *MSH2*, & *MSH6*
  - Estimate true prevalence is about 4%

Has the test been validated on all populations to which it may be offered?

- Initial studies done in Finland & Sweden
- Surveys conducted in UK and Germany
- Limited studies done in Uruguay & Argentina (Lynch, 2000)
- In US, clinical validity reported for Caucasians, but not for African Americans, Asian Americans or Hispanic Caucasians and this is a **Gap in Knowledge**.

What are the genotype/phenotype relationships?

- *MSH2* mutations have more extracolonic tumors than *MLH1*
- *MSH6* mutations have later onset & are associated with endometrial cancer
- Homozygosity for a mutation in MMR genes produces NF-1 phenotype & leukemia/lymphoma
- Mutation-negatives had CRC at later age, lower frequency multiple CRC, more involve rectum, lower frequency cancers in HNPCC-associated organs
- Turcot's syndrome: CRC and CNS tumors

What are the genetic, environmental, or other modifiers?

- Smoking is associated with MSI-H
- Increased dietary heterocyclic aromatic amines associated with MSI-H
- Estrogen use is associated with MSS
- Nonsteroidal inflammatory drugs (in culture) reduce number of cells with MSI
- Increased penetrance in males (80%) & lower in females (40%)
- Earlier age of diagnosis associated with N-acetyltransferase I allele in Finnish with *MLH1* and cyclin D1 allele

Acknowledgements

- ACCE-Mini-review:

  DNA Testing Strategies aimed at Reducing Morbidity & Mortality from Hereditary Non-Polyposis Colorectal Cancer (HNPCC)

  – Peter Rowley, James Haddow, Glenn Palomaki